論　文　内　容　要　旨

GLIS1, a novel hypoxia-inducible transcription factor, promotes breast cancer cell motility via activation of WNT5A

（新規低酸素誘導性の転写因子 GLIS1 は WNT5A の活性化を介して乳癌細胞の運動性を促進する）

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Gli-similar 1 (GLIS1) is a member of the Krüppel-like zinc finger transcription factor family, which is an interaction partner of the ligand-binding domain of the nuclear orphan receptor ROR-γ. Previous research indicates that GLIS1 plays important roles in embryonic development but not in adult tissues, it is somehow expressed in some cancer cells in which it might play distinct roles related to cancer development. We previously demonstrated that expression of GLIS1 dramatically increases under hypoxic conditions via a transcriptional mechanism induced by HIF-2α cooperating with AP-1 members. However, the roles of the hypoxia-induced GLIS1 in cancer cells remain unclear. In addition, hypoxic environment is known to play pivotal roles in breast cancer progression and hypoxia inducible factors maintain stemness in breast cancer. In this study, I thus focused on the functional roles of GLIS1 in breast cancer.

I firstly examined the effects of the reduced GLIS1 expression levels of on cell proliferation, migration and invasion capacities of BT-474 breast cancer cells which have already been shown to respond well to hypoxia stress in terms of the induction of endogenous GLIS1 gene. Knockdown of GLIS1 using siRNA in BT-474 cells resulted in significantly increased cell viability under normoxia, but did not affect viability under hypoxic conditions. On the other hand, cell-invasion capacity of siRNA BT-474 cells revealed that inhibition of GLIS1 expression with siGLIS1 drastically suppressed cell invasion under hypoxic conditions.

To clarify functional roles of upregulated GLIS1 in cancer cells, stably transfected cell lines were established that constitutively overexpress GLIS1 in MDA-MB-231 cells, in which hypoxic induction of GLIS1 was not observed. In MDA-MB-231 cells expressing FLAG-GLIS1, GLIS1 attenuated cell proliferation and enhanced cell mobility and invasion capacities under normoxic conditions.

Comprehensive gene expression analysis by RNA-sequencing of GLIS1 knockdown BT-474 cells demonstrated that GLIS1 activated 133 genes and suppressed 44 genes under normoxic conditions, whereas under hypoxic conditions GLIS1 activated 305 genes and suppressed 517 genes. Gene set enrichment analysis of those activated and suppressed genes with Metascape, a gene annotation and analysis resource, demonstrated that GLIS1 regulates various cellular mechanisms related to cell metabolic processes, interferon signals, embryonic development, morphogenesis, proliferation, differentiation, the cell cycle and response to X-ray, among others. Comprehensive gene expression analysis of MDA-MB-231 expressing FLAG-GLIS1 under normoxic conditions also identified 897 genes activated by GLIS1 and 626 genes suppressed by GLIS1. Gene set enrichment analysis further demonstrated that GLIS1 regulates various cellular functions related to cell adhesion, proliferation, differentiation, the extracellular matrix, chemotaxis and so on. Among them, WNT5A was identified as one of the target molecules related to GLIS1-induced cellular

functions; expression of WNT5A was strongly induced in MDA-MB-231 cells expressing FLAG-GLIS1, although other members of the WNT family were not altered in the RNA-sequencing result. Quantitative RT–PCR confirmed that the highest level of WNT5A induction was in MDA-MB-231 cells expressing FLAG-GLIS1, although expression of WNT2, WNT10A, WNT10B and WNT11 was also slightly altered. Knockdown experiments of WNT5A indicated that enhancement of acquired cell motility in the MDA-MB-231 cells expressing GLIS1 was mediated, at least in part, by WNT5A.

Since gene set enrichment analysis suggested that GLIS1 might suppress expression of genes related to radiation response, but in fact BT-474 cells under hypoxic conditions showed relatively greater resistance to irradiation than under normoxia, we next evaluated radiation sensitivity of the MDA-MB-231 cells expressing FLAG-GLIS1. Irradiation by γ-rays at 5 or 10 Gy effectively killed MDA-MB-231 cells transfected with control FLAG, but survival of cells expressing FLAG-GLIS1 was significantly better, confirming the importance of the GLIS1 expression in the breast cancer treatment.

To estimate prognostic significance of GLIS1 expression in breast cancers, a meta-analysis-based validation by Kaplan–Meier Plotter was performed, in which data exist on expression of 54000 genes and their association with survival in 6234 breast cancer patients. Overall survival (OS) of patients was compared between those with tumors showing high levels of GLIS1 expression and those with tumors showing low levels of GLIS1 expression. Kaplan–Meier curves indicated that expression levels of GLIS1 were not correlated with OS, either among all patients or among patients with estrogen receptor-positive cancers. On the other hand, a high level of GLIS1 expression among patients with ER-negative breast cancer was correlated with shorter OS. Furthermore, levels of GLIS1 expression in grade I or II breast cancers were not correlated with OS, but higher expression in grade III breast cancers was correlated with poorer prognosis. Level of WNT5A expression was similarly correlated with poorer prognosis, but expression levels of other members of the same gene family were not.

In this study, I found that GLIS1 plays an important role in breast cancer phenotype, i.e. cellular growth, invasiveness and migration capacities. Most importantly, higher expression of GLIS1 was found to be associated with worse prognosis in patients with estrogen receptor ER-negative breast cancer.